[2,4-DICHLOROPHENOXYACETIC ACID] Developmental Toxicity Study/Rabbit [§83-3(b)/OPPTS 870.3700

Amendment to DER for MRID 41747601 - Developmental Toxicity Study - Rabbit [2,4-D]

This amendment provides an EXECUTIVE SUMMARY and data tables to upgrade the original DER [Document No. 008462].

EPA Reviewer: Linda L. Taylor, Ph.D. Linda Lee Taylor 11/19/02
Reregistration Branch I, Health Effects Division (7509C)
EPA Secondary Reviewer: Whang Phang, Ph.D. Vihy Phang 11/21/02
Reregistration Branch I, Health Effects Division (7509C)
TXR# 005360

AMENDED DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity - rabbit § 83-3(b)/OPPTS 870.3700
P.C. CODE: 030001 REREGISTRATION CASE NO.: 818706
CASWELL NO.: 315 CAS #: 94-75-7
TEST MATERIAL (PURITY): 2,4-Dichlorophenoxyacetic acid [96.1%]
SYNONYMS: 2,4-D
SPONSOR: Industry Task Force on 2,4-D Research Data

CITATION: Hoberman, A. M. (1990). Developmental Toxicity [Embryo-Fetal Toxicity and Teratogenic Potential] Study of 2,4-Dichlorophenoxyacetic Acid [2,4-D Acid] Administered Orally *via* Stomach Tube to New Zealand White Rabbits. Argus Research Laboratories, Inc. Study No. 320-003, December 12, 1990. MRID 41747601; Document No. 008462. Unpublished.

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 41747601], artificially-inseminated female New Zealand White rabbits [20/group] were administered 2,4-Dichlorophenoxyacetic acid [96.1%] at dose levels of 0 [aqueous 0.5% methylcellulose], 10 mg/kg/day, 30 mg/kg/day, and 90 mg/kg/day from gestation day 6 through gestation day 18. NOTE: All dose concentrations were corrected for the 96.1% purity of the test material.

There were no treatment-related deaths. Two high-dose does aborted [days 21 and 24]. Treatment-related clinical signs of toxicity were observed in the two does that aborted [days 21 and 24, after 13 doses each] and included ataxia in both [days 16-19 and after day 13], and decreased motor activity, loss of righting reflex, extremities that were cold to the touch, and dried feces in doe that aborted on day 21. Body weights were comparable among the groups throughout the study, but body-weight gains were decreased at the high-dose level [73% of control] during the dosing period [days 6-19; statistical significance was not attained]. During days 7-8, the low- and mid-dose groups showed no body-weight gain, and the high-dose group displayed a negative body-weight gain [-0.01 grams] compared to the control [+0.01 gram]. During days 15-19, the high-dose group displayed no body-weight gain, and corrected body-weight gain was decreased at the high-dose level [77% of control; statistical significance was not attained] also. Food consumption was comparable among the groups.

Pregnancy rates were comparable among the groups. Comparable numbers of corpora lutea, implantations, and live fetuses were observed among the groups, and there were no dead fetuses. One control doe had 100% resorptions. The number of resorption and pre- and post-implantation losses were comparable among the groups also. Gravid uterine weights were comparable among the groups.

Mean fetal body weight was comparable among the groups. At the high-dose level, there was a significant increase in the percent of live male fetuses [71.2%] compared to the control [52.8%] and other dose groups [low: 54.4%; mid: 59.4%]. At the high-dose level, the fetal incidence [3 fetuses of one litter; $p<0.01$] of hindlimbs turned inward was increased compared to the control (0) and other treatment groups (0), and the same fetuses displayed domed head [hydrocephaly]. This finding is not considered treatment-related. There were no apparent differences in the incidence of external, visceral, or skeletal variations, anomalies, retardations, or malformations among the groups.

The maternal toxicity NOAEL is 30 mg/kg/day, based on abortions, decreased body-weight gain, and clinical signs of toxicity [decreased motor activity, ataxia, loss of righting reflex, extremities cold to the touch] at the maternal toxicity LOAEL of 90 mg/kg/day.

The developmental toxicity NOAEL is 30 mg/kg/day, based on abortions at the developmental toxicity LOAEL of 90 mg/kg/day.

This developmental toxicity study is classified ACCEPTABLE/Guideline, and it satisfies the guideline requirement **[§83-3(b)/OPPTS 870.3700]** for a developmental toxicity study in rabbits.

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[2,4-DICHLOROPHENOXYACETIC ACID] Developmental Toxicity Study/Rabbit [§83-3(b)/OPPTS 870.3700]

Table 1. Maternal Body Weight/Body-Weight Gain [kilograms]				
Dose/Time	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
Body Weight [kg]				
gestation day 0	3.16±0.23	3.19±0.28	3.15±0.25	3.17±0.24
gestation day 7	3.36±0.25	3.38±0.29	3.34±0.29	3.38±0.25
gestation day 14	3.50±0.26	3.51±0.31	3.52±0.30	3.52±0.27
gestation day 20	3.59±0.27	3.58±0.30	3.56±0.30	3.54±0.32
gestation day 29	3.82±0.30	3.80±0.32	3.82±0.34	3.76±0.28√
# pregnant	17	18	16	18
Body-Weight Gain [kg]				
days 0-6	0.19±0.07	0.18±0.04	0.17±0.06	0.19±0.07
days 6-7	0.01±0.03	0.01±0.04	0.02±0.05	0.01±0.05
days 7-8	0.01±0.04	0.00±0.03	0.00±0.03	-0.01±0.05
days 9-12	0.05±0.04	0.04±0.05	0.09±0.04	0.06±0.04
days 15-19	0.05±0.04	0.05±0.03	0.05±0.06	0.00±0.15
days 6-19	0.22±0.08	0.22±0.07	0.24±0.08	0.16±0.21 [73]♪
days 6-29	0.47±0.12	0.43±0.12	0.49±0.12	0.42♪0.11√ [89]
days 0-29	0.66±0.14	0.61±0.13	0.66±0.14	0.62±0.12√
days 6-29C	0.03±0.16	0.02±0.16	0.06±0.11	-0.03±0.12√
days 0-29C	0.22±0.17	0.20±0.16	0.23±0.13	0.17±0.13√ [77]
Gravid Uterine Wt. [g]	439.89±129.43	405.72±98.12	430.98±106.06	448.66±122.63

√ excludes 2 does that aborted; ♪ [% of control]; C corrected for gravid uterine weight
Data from Tables 4-5, pages 47-50 of the report

Table 2. Food Consumption				
interval [days]	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	90 mg/kg/day
grams/day				
0-6	175.9±12.0	177.8±11.5	178.4±7.7	180.4±4.8
6-7	172.2±20.4	168.9±32.0	174.0±16.0	177.8±11.5
7-8	174.5±19.5	167.8±21.7	172.2±22.2	167.4±45.8
6-9	172.6±19.2	166.6±28.4	176.8±11.8	171.6±29.5
15-19	170.6±21.2	172.1±17.8	174.9±14.5	160.3±50.2
6-19	169.3±16.5	166.1±22.4	174.2±14.5	166.9±26.4
0-29	164.1±18.2	161.8±18.1	169.6±16.0	165.1±12.0√
grams/kg/day				
0-6	54.1±3.4	54.3±3.4	55.3±3.7	55.2±3.4
6-7	51.3±5.3	50.1±9.0	52.6±6.9	53.0±5.6
7-8	31.8±4.1	49.8±6.1	51.8±7.4	49.6±14.3
6-9	51.2±4.3	49.1±7.5	53.3±5.0	50.3±8.8
15-19	48.0±5.3	48.4±3.4	49.2±5.2	45.3±14.3
6-19	48.9±3.6	47.9±5.0	50.3±5.3	48.4±7.9
0-29	46.2±3.7	45.9±3.8	48.3±5.0	47.1±3.2

√ excluded 2 does that aborted; data from Tables 6-7, pages 51-54 of the report

008462

GUIDELINE: 83-3

Primary Review by: Karen E. Whitby, Ph.D. Karen Whitby 7/3/91
Toxicologist, Review Section II, Toxicology Branch II/HED (H7509C)

Secondary Review by: K. Clark Swentzel K. Clark Swentzel 7/3/91
Section Head, Review Section II, Toxicology Branch II/HED (H7509C)

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: Rabbit
Guideline: 83-3

EPA Identification No.s: EPA MRID (Accession) No.:417476-01
Caswell No.: 315
HED Project No.: 1-0621

Test Material: 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid)

Sponsor: Industry Task Force on 2,4-D Research Data

Study Number(s): 320-003 (Argus Research Laboratories)

Testing Facility: Argus Research Laboratories, Inc.
935 Horsham Road
Horsham, Pa. 19044

Title of Report: Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid) Administered Orally via Stomach Tube to New Zealand White Rabbits

Author(s): Alan M. Hoberman, Ph.D.

Report Issued: December 12, 1990 (Final Report)

Study Dates: April 2, 1990 (first insemination)
May 4, 1990 (last cesarean section)

Bibliographic Citation: Hoberman, A.M., (1990) Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid) Administered Orally via Stomach Tube to New Zealand White Rabbits. Study Number(s): 320-003 (Testing Facility) Argus Research Laboratories, Inc. 935 Horsham Road Horsham, Pa. 19044

Conclusions:

The test substance, 2,4-D Acid was administered by gavage to presumed pregnant rabbits at 0, 10, 30, and 90 mg/kg/day in 0.5% methylcellulose. Maternal toxicity was observed in two high dose does with ataxia. One high dose doe that aborted on day 21 had decreased bodyweight, food consumption, dried feces, ataxia, loss of righting reflex, decreased motor activity, extremities that were cold to the touch, and findings at necropsy. There was a slight nonsignificant reduction in bodyweight gain during the dosing and postdosing periods and a nonsignificant reduction in corrected bodyweight gain during the entire period for the high dose group. There were no significant or dose related alterations in fetal development that could be attributed to treatment. The fetal incidence [3(1)] of hindlimbs turned inward was significantly increased ($p \leq 0.01$) at 90 mg/kg. Also at 90 mg/kg, the fetal and litter incidence of domed head (and therefore, hydrocephaly) was equal to that for hindlimbs turned inward relative to the control.

Core Classification: Core-Minimum

Maternal NOEL = 30 mg/kg/day
Maternal LOEL = 90 mg/kg/day
Developmental Toxicity NOEL = 90 mg/kg/day
Developmental Toxicity LOEL = >90 mg/kg/day

RANGE-FINDING STUDY

The main teratogenicity study was preceded by a pilot study, which was performed to determine the dosage levels to be used in the main study. The study was sponsored by Industry Task Force on 2,4-D Research Data, and was performed by Argus Research Laboratories, Inc..

Study Title: Dosage-Range Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of 2,4-Dichlorophenoxyacetic Acid (2,4-D Acid) Administered Orally via Stomach Tube to New Zealand White Rabbits (Pilot Study)

Study No.: Argus Research Laboratories, Inc., Protocol 320-003P

Date of Report: May 11, 1990

Date of Study: November, 2, 1989 (Insemination)

Author: Alan Hoberman, Ph.D. (Study Director)

Materials and Methods

The test substance 2,4-D Acid was administered by gavage days 6 through 18 of gestation at 0 (vehicle), 12.5, 25, 50, 100, and 200 mg (4 ml/kg/day, adjusted daily for bodyweight). The vehicle was 0.5% (w/w) methylcellulose. The doses were corrected for the 96.1% acid equivalent of the test substance. There were 4 animals per treatment group. On day 29 the animals were euthanized and necropsied. Corpora lutea were counted and the livers and kidneys weighed. The gravid uterus was excised and weighed. The number and location of implantation sites, early and late resorptions, and live and dead fetuses were recorded. Fetuses were weighed sexed, and examined for gross alterations.

Results

One doe in the 100 mg/kg group aborted and was sacrificed on day 22. Another doe in this group was sacrificed in the moribund condition. At the 200 mg/kg level, three does died. The does that were found dead, sacrificed, or aborted were found to have weight loss and decreased food consumption, impaired or lost righting reflex, decreased motor activity, ataxia, dyspnea, coldness to the touch, and/or a red substance present in the anogenital area or cage pan. A red-brown viscous fluid or orange-red fluid was present in the urinary bladder of the does that were found dead or sacrificed. With the exception of the does that aborted, were sacrificed moribund, or found dead there were no alterations in clinical signs, bodyweight or food consumption. There was no effect on maternal kidney weights. Absolute and relative liver weight was increased for the one doe surviving to the end of the

study. The litter of the one surviving doe at 200 mg/kg consisted of one viable fetus and 8 early resorptions, and the litter of the 100 mg/kg doe that aborted consisted of three early resorptions. All fetuses appeared normal at gross external examination.

Conclusions:

The dosage levels selected for the main study were 0, 30, 60, and 90 mg/kg.

A. Materials

A copy of the "materials and methods" section from the investigators report is appended.

Test Compound:

The test article was stored at room temperature. Suspensions of the test article were prepared daily [as 0 (vehicle), 2.5, 7.5, and 22.5 mg/mL) and administered in a volume of 4 mL/kg. All dose concentrations were corrected for the 96.1% activity (purity) of the test substance.

The report references the pilot study for the homogeneity, concentration, and stability analyses. In addition, the report indicates that two 5 g samples of the test substance were sent to the sponsor for possible analyses, one at the beginning and one at the end of the dosing period. The results of this analyses are available in the sponsor's records.

Description:	tan granular powder
Lot No.:	909

Vehicle(s):

The vehicle was aqueous 0.5% methylcellulose (Sigma Chemical Co., Lot 88F0051). The vehicle was received in the form of white powder and stored at room temperature. The vehicle was prepared in R.O. deionized water. The water was analyzed for chemical and bacterial contaminants. After preparation, the vehicle was stored under refrigeration.

Test Animal(s):	Species:	Rabbit
	Strain:	New Zealand White [Hra:(NZW)SPF]
	Source:	Hazleton Research Products, Inc. Denver, PA
	Age:	approx. 5 mo. (upon receipt)
	Weight:	2.21 - 3.73 kg the day after arrival

B. Study Design

This study was designed to assess the developmental toxicity potential of 2,4-D Acid when administered orally by stomach tube to female New Zealand White rabbits on gestation days 6 through 18, inclusive.

Mating

The females were artificially inseminated with semen from proven male breeders of the same strain, obtained from the same source. Each of these rabbits were given an i.v. injection of 20 USP Units of HCG (PREGNYL, Organon, Inc., Lot 0130289315) approx. 3 hrs.

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prior to insemination. Semen was diluted with normal saline (Abbott Laboratories, Inc., Lot 34-201-JT) to achieve a concentration of 6.0 X 10^6 spermatozoa/0.25 mL for insemination. The day of insemination was considered day 0 of gestation. One quarter of the rabbits assigned to each group were inseminated each day. Four proven male breeders inseminated each group of females.

Group Arrangement:

One hundred four virgin female rabbits were received from the supplier. One animal upon exam by the Staff Veterinarian was found to be unacceptable. Another rabbit had an apparent fracture of the right rear leg and was sacrificed prior to assignment to the study. Eighty of these animals were assigned to the study (20/group) using a computer-generated weight ordered randomization procedure, after a 3 week acclimation period. The remaining 22 were reassigned to the general population.

Test Group	Dose Level (mg/kg)	Number Assigned
Vehicle Control	0	20
Low Dose	10	20
Mid Dose	30	20
High Dose	90	20

NOTE: Animal 16888 (vehicle control) was not dosed on day 9 due to repeated difficulties in placing the stomach tube. The technician deemed it necessary to refrain from further attempts so as to not jeopardize the health and life of the animal.

Animal Husbandry:

The study room had 370 sq. ft. of floor space and a minimum of 10 changes per hour of 100% fresh HEPA filtered (99.97%) air. The room temperature was 64 - 74° F; humidity was 35 - 65% during the study period. The animals were maintained on a 12 hour light/dark cycle. The animals were fed 180 g Purina Certified Rabbit Chow® # 5322. Local water that had passed through a R.O. membrane was also available ad lib via glass water bottles. Chlorine had been added to the water as a bacteriostat. The water samples contained from 0.0 to 0.6 ppm of chlorine.

Dosing:

The doses used in this investigation were selected on the basis of a dose range finding study.

All doses were in a volume of 4 mL/kg of bodyweight/day (adjusted daily for bodyweight). Suspensions of the dosing solutions were prepared daily. Triplicate 10 mL samples of each concentration

were reserved from the first and last time the dosage solution were prepared. Two of the samples were frozen and sent to Lancaster Laboratories, Inc. for analysis. The third frozen sample was kept at the facility as a backup. Two 5 g samples of the bulk test substance were also shipped to Lancaster Laboratories for possible analysis.

The dosing solutions for the pilot and main study were analyzed by Lancaster Laboratories. The detection limit was <0.05 mg/g. The results for the main study are as follows:

Theoretical Concentration of Dosing Solution:	Actual Range:
0 mg/mL	0
2.5 mg/mL	3 to 6%
7.5 mg/mL	3 to 4%
22.5 mg/mL	5 to 8%

The protocol indicates that the bulk test substance is stable at room temperature (stability of the test substance is on file with the sponsor).

Observations

Animals were observed for clinical signs several times during the acclimation period and on day 0 of pregnancy. The animals were checked for mortality at least twice daily. Clinical observations were performed three times each day during the dosing period (days 6-18), and once a day during the post dosing period.

Bodyweight was recorded on day 0, and days 6 through 29 of gestation. Food consumption was recorded daily.

Does were sacrificed on day 29 of gestation by i.v. administration of T-61® Euthanasia Solution. Thoracic and abdominal cavities were examined for gross lesions. In the event of gross lesions (except for parovarian cysts which are common in rabbits) the tissues were preserved in neutral buffered 10% formalin. A laparotomy was performed and the intact uterus was excised and weighed. Uteri that appeared nonpregnant were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, the number and placement of implantation, early and late resorptions, and live and dead fetuses were recorded. Each fetus was removed from the uterus and individually identified with a tag, weighed, and observed for gross external alterations. Viable fetuses were sacrificed. Every fetus was examined to determine sex and soft tissue alterations. The brain was free-hand sectioned, and examined. Fetuses were then eviscerated, stained with Alizarin red S, and examined for skeletal changes. Skeletal specimens were stored in 80% glycerine with thymol crystals to retard fungal growth. Photographs were taken of all abnormal findings.

Historical control data were provided to allow comparison with concurrent controls.

Statistical Analysis

The section on statistical analyses is appended.

Compliance

A signed Statement of No Confidentiality Claim was provided dated December 15, 1990 (p 2).

A signed Statement of Compliance with EPA GLP's was provided that was dated December 15, 1990 (p 3).

A signed Quality Assurance Statement was provided dated December 12, 1990 (pp. 475 to 478).

A signed statement from the Chairman of the Technical Committee Industry Task Force II on 2,4-D Research Data, dated December 15, 1990 was provided, which indicated the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects were applied to this study. This study neither reportedly met or exceeded any of these criteria.

C. Results

1. Maternal Toxicity

Mortality

With the exception of two nonpregnant control does, all females survived to their scheduled sacrifice. One of the does was presumed to have died (on day 12) as a consequence of an intubation accident. Necropsy of the doe revealed hemorrhagic lungs. The cause of death for the other doe was not determined.

Clinical Observations

Alterations in clinical observations which were attributable to the test substance were observed in two high dose does. One doe (16937) had ataxia on days 16 to 19. Doe 16944 aborted on day 21. This doe presented the following clinical findings: dried feces, ataxia, decreased motor activity, loss of righting reflex, and extremities that were cold to the touch. Weight loss was observed in this animal after day 13. Feed consumption was decreased after day 5, decreases were more marked after day 12. Necropsy revealed a red substance in the anogenital area, a large gallbladder, and parovarian cysts.

Alopecia occurred in 2, 0, 3, and 2 does in the control, low, mid, and high dose groups. Rales occurred for 2, 3, 1, and 0 does in

the control, low, mid, and high dose groups. One control group doe had a red substance in the in the cage pan and was noted at necropsy to have a fluid filled uterus (the litter of this doe consisted of four early resorptions).

Bodyweight

Table I: Bodyweight Gains (kg)[a]

Dose (mg/kg):	Days 0-6	Days 6-19	Days 19-29	Days 0-29	Corrected Body Weight Gains Dosing P.[1]	Entire[2]
0	0.19	0.22	0.25	0.66	-0.22	0.22
10.0	0.18	0.22	0.22	0.61	-0.19	0.20
30.0	0.17	0.24	0.25	0.66	-0.19	0.23
90.0	0.19	0.16	0.21	0.62	-0.24	0.17

1 = bodyweight gain during dosing period minus gravid uterus weight.
2 = corrected bodyweight = day 29 gestation bodyweight minus gravid uterine weight
a = Data extracted from (study number 320-003 tables 5 and 17 pp. 49 and 78-89)
(Some of the above values were calculated by this reviewer.)

The only time during which a reduction in maternal bodyweight gain was noted was during the dosing period (most notably during the initial phase of dosing). The average maternal weight gain was 100, 109.1, and 72.7% of the control value for the low, mid, and high dose groups, respectively. During the post-dosing period, the average maternal bodyweight gains were 88, 100, and 84% of the control values for the low, mid, and high dose groups. For the same respective groups the corrected bodyweight gain during the dosing period was 86.4, 86.4, and 109.1% of the control values; and the corrected bodyweight gain during the entire gestation period was 90.9, 104.5, and 77.3% of the control value.

Food Consumption

There were no significant differences in absolute (g/day) or relative (g/kg/day) maternal food consumption. Food consumption data excludes values for wet or spilled feed. Values for the does that aborted at the highest dose group were also excluded from the data.

Table II: Food Consumption Data (g/kg/day)[a]

Dose (mg/kg)	Days 0-6	Days 6-19	Days 19-29	Days 0-29
0	54.1	48.9	40.4	46.2
10.0	54.3	47.9	39.2	45.8
30.0	55.3	50.3	43.4	48.3
90.0	55.2	48.4	40.4	47.1

a = Data extracted from (study number 320-003 table 7 p.53)

Gross Pathological Observations

None of the necropsy findings were considered to be related to treatment. Some of the findings noted at necropsy have been presented above. Doe 16936 (of the high dose group) that aborted on day 24 was found to have mottled lungs. Doe 16944 (of the high dose group) that aborted on day 21 and was found to have a red substance present in the anogenital area also was found to have a large gall bladder and a parovarian cyst. Another doe of the high dose group was found to have the abdominal and thoracic cavities filled with clear fluid. Additional necropsy findings included parovarian cysts among all treatment groups.

Cesarean Section Observations

The does that aborted in the HDT were excluded from cesarean section observations. Table 9 of the report (p.56) indicates that the % resorbed conceptuses/litter was 6.4 for the control group. Apparently the value for doe 16876 was omitted from this mean. This doe had a litter that consisted of four early resorptions (litter 100% resorbed), which the author states is a relatively common event in rabbits. Historical control data is provided to support this statement.

There was a significant (dose-related) increase in the average percentage of live male fetuses in the 90 mg/kg group. The author reports that this value was even greater than that observed historically. When the author excluded litters containing only one sex, this finding was not statistically significant or dose-dependent.

No other findings were significantly altered relative to the control.

Table III: Cesarean Section Observations[a]

Dose (mg/kg):	0	10	30	90
#Animals Assigned	20	20	20	20
#Animals Inseminated	20	20	20	20
# (%) Pregnant	17(85.0)	18(90.0)	16(80.0)	18(90.0)
N	17	18	16	16
Maternal Wastage				
#Died	2	0	0	0
#Non pregnant	3	2	4	2
#Aborted	0	0	0	2
#Premature Delivery	0	0	0	0
Total Corpora Lutea	171	178	158	163
Corpora Lutea/Dam	10.0	9.9	9.9	10.2
Total Implantation	132	124	125	129
Implantation/Dam	7.8	6.9	7.8	8.1
Total Live Fetuses	119	120	109	116
Live Fetuses/Dam	7.0	6.7	6.8	7.2
Total Resorptions	13	4	16	13
Early	9	3	11	13
Late	4	1	5	0
Resorptions/Dam	0.8	0.2	1.0	0.8
Total Dead Fetuses	0	0	0	0
Dead Fetuses/Dam	0	0	0	0
Mean Fetal Weight (g)	45.13	46.14	45.05	44.43
♂ fetuses	45.32	44.62	45.42	44.57
♀ fetuses	44.21	46.34	43.82	43.81
Preimplantation Loss(%)	22.5	29.6	19.4	19.3
Postimplantation Loss(%)	11.9	3.8	11.5	10.4
Sex Ratio (% Male)	52.8	54.4	59.4	71.2*

a Data extracted from (study number 320-003; tables 8, 9 pp. 55, 56 and tables 19 and 20 pp.102-109)

* Significantly different from vehicle control ($p \leq 0.05$)

(Some of the above values were calculated by this reviewer.)

2. Developmental Toxicity

Table IV: External Examinations

Dose (mg/kg):	0	30	60	90
Observations*				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
Head:				
Domed	0(0)	1(1)	0(0)	3(1)
Rhinocephaly	0(0)	1(1)	0(0)	0(0)
Palate:				
Cleft, Medial	0(0)	0(0)	0(0)	1(1)
Body:				
Kyphosis	0(0)	0(0)	0(0)	1(1)
Umbilical Hernia	0(0)	1(1)	0(0)	1(1)
Pale	0(0)	0(0)	0(0)	1(1)
Forelimb(s):				
Turned Inward	0(0)	0(0)	0(0)	2(1)
Paw, Left, Clenched	0(0)	0(0)	1(1)	0(0)
Hindlimb(s):				
Turned Inward	0(0)	0(0)	0(0)	3*(1)

Data extracted from (study number 320-003; table 11 pp. 58-59, and table 22 pp.118-149)
+ = some observations may be grouped together
a = fetal (litter) incidence
* = significantly different from control ($p \leq 0.01$)

The fetal incidence of hindlimbs turned inward was significantly increased at 90 mg/kg. The fetal and litter incidence of domed head (and therefore, hydrocephaly) relative to the control was equal to that for hindlimbs turned inward also at 90 mg/kg. Fetus 16909-5 (of the 10 mg/kg group) had rhinocephaly, proboscis like nose above partially fused eyes and additional observations made upon soft tissue and skeletal exam. Fetus 16937-1 had a dome shaped head, a left turned fore- and hindlimb and other abnormalities observed with the soft tissue and skeletal exam. Fetus 16937-3 had a dome shaped head; the hindlimbs were bilaterally turned inward and other abnormalities observed with the soft tissue and skeletal exam. Fetus 16937-6 had a dome shaped head, medial cleft palate, kyphosis, fore- and hindlimbs were bilaterally turned inward and other abnormalities observed with the soft tissue and skeletal exam.

Table IV: Visceral Examinations

Dose (mg/kg):	0	10	30	90
Observations				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
Brain:				
Extreme Dilation of Lateral Ventricles (Hydrocephalus)	0(0)	1(1)	0(0)	3(1)
Protrudes	0(0)	1(1)	0(0)	0(0)
Lungs:				
Intermediate Lobe, Agenesis	2(2)	7(5)	3(2)	5(2)
Gallbladder:				
Smaller than Normal	2(2)	0(0)	0(0)	3(3)
Larger than Normal	0(0)	0(0)	1(1)	1(1)
Liver:				
Protrudes	0(0)	1(1)	0(0)	1(1)

Data extracted from (study number 320-003; table 12 pp. 61-62 and table 22 pp.118-149)
+ = some observations may be grouped together
a = fetal (litter) incidence

Upon soft tissue exam, fetus 16891-7 of the 10 mg/kg group, was found to have agenesis of the intermediate lobe of the lung and protruding liver. This fetus also exhibited findings in the skeletal exam. The three fetuses of the same litter (16937) that were observed to have domed head in the external exam were found to have hydrocephalus in the soft tissue exam.

Table IV: Skeletal Examinations

Dose (mg/kg):	0	10	30	90
Observations*				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
SKULL:				
Irregular Ossification	45(14)	42(17)	40(13)	45(15)
Nasal(s), Irregular Ossification:				
Internasal	0(0)	2(1)	1(1)	1(1)
Intranasal	0(0)	2(2)	3(3)	2(1)
Irregular Suture	4(3)	0(0)	0(0)	2(2)
Midline Suture Displaced	20(10)	16(10)	11(9)	22(11)
Small &/or Irregularly Shaped	0(0)	1(1)	1(1)	0(0)
Nasal(s)-Frontal(s) Irregular Suture	1(1)	0(0)	3(2)	3(3)
Frontal(s), Irregular Ossification:				
Interfrontal	3(3)	5(3)	4(3)	3(3)
Intrafrontal	2(2)	2(2)	4(4)	2(2)
Irregular Suture	26(11)	22(13)	20(12)	17(9)
Fused	0(0)	1(1)	0(0)	0(0)
Small and Irregularly Shaped	0(0)	1(1)	0(0)	0(0)
Parietal, Intraparietal	0(0)	1(1)	1(1)	0(0)
Zygomatics, Appear Broad	0(0)	1(1)	0(0)	0(0)
Maxillas, Short & Close-Set	0(0)	1(1)	0(0)	0(0)
Premaxillas, Absent	0(0)	1(1)	0(0)	0(0)
Palate, Inc. Ossified	0(0)	0(0)	0(0)	1(1)
Eye Sockets, Formed as One Below the Nasal	0(0)	1(1)	0(0)	0(0)
Anterior & Posterior Fontanelles Enlarged (Moderate)	0(0)	0(0)	0(0)	2(1)
Anterior & Posterior Fontanelles Enlarged (Marked)	0(0)	1(1)	0(0)	1(1)
Anterior Fontanelle, Additional Ossif.	0(0)	1(1)	0(0)	0(0)
Parietals, Contain Small Holes	0(0)	1(1)	0(0)	2(1)
Frontals, Contain Small Holes	0(0)	1(1)	0(0)	0(0)
HYOID, Ala(e), Angulated	4(3)	3(3)	4(3)	5(3)

Table IV: Skeletal Examinations- cont'd

Dose (mg/kg):	0	10	30	90
Observations*				
#pups(litters) examined	119(16)	120(18)	109(16)	116(16)
VERTEBRAE:				
Cervical:				
Centrum Unilateral Ossification	0(0)	1(1)	0(0)	0(0)
Arch, Absent	0(0)	1(1)	0(0)	0(0)
Thoracic:				
Hemivertebra	0(0)	1(1)	0(0)	1(1)
Centrum, Unilateral Ossification	0(0)	1(1)	0(0)	1(1)
Lumbar:				
Hemivertebra	0(0)	0(0)	1(1)	0(0)
Caudal:				
Misaligned	1(1)	1(1)	1(1)	1(1)
INTERRELATED VERTEBRAL/ RIB MALFORMATIONS	0(0)	1(1)	0(0)	2(1)
RIBS:				
Fused	0(0)	1(1)	0(0)	3(1)
Split	0(0)	2(1)	0(0)	2(1)
Thickened	3(3)	0(0)	1(1)	0(0)
Wavy	0(0)	0(0)	0(0)	1(1)
STERNEBRAE:				
Fused	0(0)	1(1)	1(1)	0(0)
SCAPULAE:				
Alae, Irregularly-Shaped	0(0)	1(1)	0(0)	0(0)

Data extracted from (study number 320-003; table 13 pp. 63-69 and table 22 pp.118-149)
+ = some observations may be grouped together
a = fetal (litter) incidence

Table 14, page 70 of the report indicates that there was a significant increase ($p \leq 0.01$) in the mean number of ossification sites in the lumbar vertebrae, and a significant decrease ($p \leq 0.01$) in the number of thoracic vertebrae at the 10 mg/kg level. Also at this level there was a significant reduction ($p \leq 0.05$) in the mean number of rib pairs. The report indicates that the author's facility identifies the number of thoracic vertebrae on the basis of the number of thoracic ribs present. Therefore, this observation was apparently related to the vertebral finding discussed above. Because these findings were not dose related and the incidences are within the historical control range, this observation is apparently unrelated to treatment.

The three fetuses in the high dose group of the same litter that were observed to have hydrocephalus and limbs that were turned inward were found to have enlarged anterior and posterior fontanelles. The parietals of two of these fetuses were found to contain small holes. The palate of the fetus found to have cleft palate was observed to be incompletely ossified. In addition, the ribs of this fetus bilaterally 1-12 were wavy and flat. Kyphosis in this fetus was confirmed upon the skeletal exam.

D. Discussion/Conclusions

a. Maternal Toxicity:

Two high dose does had ataxia. One high dose doe that aborted on day 21 had decreased bodyweight, food consumption, dried feces, ataxia, loss of righting reflex, decreased motor activity, extremities that were cold to the touch, and findings at necropsy. There was a slight nonsignificant reduction in bodyweight gain during the dosing and postdosing periods and a nonsignificant reduction in corrected bodyweight gain during the entire period for the high dose group.

b. Developmental Toxicity:

i. Deaths/Resorptions:

There were no significant increases in fetal deaths or resorptions in any of the treatment groups.

ii. Altered Growth:

There were no significant alterations in fetal growth observed.

iii. Developmental Anomalies:

There was no increased incidence of developmental anomalies observed in this study that may be attributed to treatment.

iv. Malformations:

The fetal incidence [3(1)] of hindlimbs turned inward was significantly increased ($p \leq 0.01$) at 90 mg/kg. Also at 90 mg/kg, the fetal and litter incidence of domed head (and therefore, hydrocephaly) was equal to that for hindlimbs turned inward relative to the control.

E. Core Classification: Core-Minimum

Maternal NOEL = 30 mg/kg/day
Maternal LOEL = 90 mg/kg/day
Developmental Toxicity NOEL = 90 mg/kg/day
Developmental Toxicity LOEL = >90 mg/kg/day